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(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR

(57) Abstract: The invention provides a method of identifying an MCH receptor agonist or antagonist, by contacting an MCH receptor with one or more candidate compounds under conditions wherein the MCH receptor produces a predetermined signal in response to an MCH receptor agonist, and identifying a compound that alters production of the predetermined signal. The invention also provides a method of identifying an MCH receptor ligand, by contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding between the MCH receptor and an MCH receptor ligand, and identifying a compound that selectively binds to the MCH receptor. Also provided are methods of identifying an individual having or susceptible to an MCH receptor-associated condition, by detecting MCH receptor nucleic acid or polypeptide in a sample. The invention further provides signaling compositions, which contain a recombinantly expressed MCH receptor and a recombinantly expressed G $\alpha$  subunit of a G protein, or which contain a recombinantly expressed MCH receptor, a G protein, and a calcium indicator.

**MELANIN CONCENTRATING HORMONE RECEPTOR****BACKGROUND OF THE INVENTION**

The present invention relates generally to the field of medicine and, more specifically, to therapeutic and diagnostic methods and compositions related to 5 melanin concentrating hormone receptor.

Obesity, or excess deposition of body fat, represents a primary health concern in industrialized nations. Obesity correlates with and may trigger the 10 onset of serious medical conditions, including hypertension, diabetes, cardiovascular disease and psychological maladjustments. Whereas diet, exercise and appetite suppressants can produce modest results in the reduction of body fat deposits, no consistently effective 15 or practical treatment has been found for controlling obesity and its physiological and psychological consequences.

Pathologically decreased body weight, or cachexia, which commonly occurs in chronic diseases such 20 as cancer and AIDS, is also a serious health concern. The weight loss characteristic of cachexia has been associated with several contributing factors, including food aversion due to altered sensitivity to taste and smell, malfunction of the gastrointestinal tract, 25 insufficient nutrient intake, and metabolic disturbances.

Melanin-concentrating hormone, or MCH, is a small, cyclic neuropeptide that plays an important role in regulating body weight, metabolism, and feeding behavior. MCH was first isolated from the pituitary 30 gland of salmon, where it functions to regulate scale color. Intracerebral administration of MCH peptide in

mammals has been shown to produce a dose-dependent stimulation of food intake, whereas mice deficient in MCH exhibit decreased body weight due to reduced feeding behavior and an inappropriately increased metabolic rate.

5 Expression of MCH is increased in the *ob* mouse model of obesity as well as in normal animals following fasting. Thus, it is clear that MCH plays a critical role in regulating body weight, metabolism and appetite.

In mammals, the pattern of MCH expression in  
10 the brain is also consistent with MCH playing a role in regulating complex behavior and in controlling the hypothalamic-pituitary-adrenal axis. Administration of MCH to rats has also been shown to regulate behavior, such as increasing female sexual receptivity, increasing  
15 anxiety, and antagonizing the effect of  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) on aggression and exploratory behavior.

In view of the important role of MCH in regulating body weight, behavior, and general neural and  
20 endocrine functions, it would be beneficial to develop compounds that mimic or antagonize MCH activity. These compounds could be used as therapeutics in conditions in which abnormal body weight, behavior, or neural and endocrine functions play a role. However, the cell  
25 surface receptor that binds MCH, and the signal transduction pathway initiated by receptor binding, have not previously been identified. Therefore, it has not been possible to develop rapid and reliable methods of screening for therapeutic compounds that can be used to  
30 regulate or alter MCH-mediated physiological or pathological functions.

Thus, there exists a need to identify the MCH receptor and to develop methods of screening for compounds that bind to MCH receptor or mimic or antagonize MCH activity. The present invention satisfies 5 this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides a method of identifying an MCH receptor agonist or antagonist. The method consists of contacting an MCH receptor with one or more 10 candidate compounds under conditions wherein the MCH receptor produces a predetermined signal in response to an MCH receptor agonist. A candidate compound that alters production of the predetermined signal is identified. The compound is characterized as an MCH 15 receptor agonist or antagonist.

The invention also provides a method of identifying an MCH receptor ligand. The method consists of contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding 20 between the MCH receptor and an MCH receptor ligand. A compound that selectively binds the MCH receptor is identified. The compound is characterized as an MCH receptor ligand.

Also provided are methods of identifying an 25 individual having or susceptible to an MCH receptor-associated condition. In one embodiment, the method consists of detecting MCH receptor nucleic acid molecule in a sample from the individual. Abnormal structure or expression of the MCH receptor nucleic acid 30 molecule in the sample indicates that the individual has or is susceptible to an MCH receptor-associated

condition. In another embodiment, the method consists of detecting MCH receptor polypeptide in a sample from the individual. Abnormal expression or activity of the MCH receptor polypeptide in the sample indicates that the  
5 individual has or is susceptible to an MCH receptor-associated condition. MCH receptor-associated conditions include disorders of body weight, mood, memory, learning, sleep, dopaminergic system function, reproduction or growth.

10 The invention also provides signaling compositions. In one embodiment, the signaling composition contains a recombinantly expressed MCH receptor and a recombinantly expressed G $\alpha$  subunit of a G protein. In another embodiment, the signaling  
15 composition contains a recombinantly expressed MCH receptor, a G protein, and a calcium indicator.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of  
20 human melanin-concentrating hormone receptor (GPR24 or SLC-1) (Kolakowski et al., FEBS Letters 398:253-258 (1996), GenBank accession number U71092).

Figure 2 shows the nucleotide sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of  
25 rat melanin concentrating hormone receptor (SLC-1) (Lakaye et al., Bioc. Biophys. Acta 1401:216-220 (1998), GenBank accession number AF008650).

Figure 3 shows purification of SLC-1 endogenous ligand from rat brain extracts. Figure 3A shows a C18  
30 reverse-phase HPLC elution profile. Figure 3B shows a

kinetic of the  $[Ca^{2+}]_i$  changes evoked by fraction 57, with or without trypsin treatment. Figure 3C shows final purification of active peptide by Sephasil C8 SC2.1/10 using SMART system. The inset panel shows the peak 5 increments in  $[Ca^{2+}]_i$  induced by designated HPLC fractions.

Figure 4 shows the specificity of interaction between MCH and SLC-1. Figure 4A shows the alignment of rat/human MCH sequence with salmon MCH, somatostatin 14 and cortistatin 14. Figure 4B shows  $[Ca^{2+}]_i$  changes in CHO cells transfected with SLC-1 and G $\alpha$ q/i3 induced by MCH, salmon MCH, somatostatin-14 (SST-14), cortistatin-14 (CS-14),  $\alpha$ -MSH, NEI, and MGOP-14. Figure 4C, left, shows dose-response curves for changes in  $[Ca^{2+}]_i$  induced by 10 SLC-1 alone or SLC-1 coexpressed with G $\alpha$ q/i3. Figure 4C, right, shows inhibition of forskolin-stimulated cAMP 15 accumulation in CHO cells transfected with SLC-1 alone.

Figure 5 shows the distribution of SLC-1 mRNA. Figure 5A shows Northern blot analysis from indicated rat 20 tissues using an SLC-1 cDNA probe (top panel) and G3PDH control probe (bottom panel). Figure 5B shows localization of SLC-1 transcripts in rat brain sections by *in situ* hybridization. Ctx : cortex; AON: anterior olfactory nucleus; TT: taenia tecta; Tu: olfactory 25 tubercle; Acb: nucleus accumbens; Pir: piroform cortex; Hpx: hippocampus; Th: thalamus; Hyp: hypothalamus; Amy: amygdala; LC: locus coeruleus.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the 30 identification of the receptor for melanin concentrating hormone (MCH) and its signal transduction pathway. The

invention thus provides novel compositions and methods that can be used to identify compounds that specifically bind to or modulate signaling through the MCH receptor. Such compounds can be used therapeutically to prevent or 5 ameliorate MCH receptor-associated conditions, including disorders of body weight, behavior, memory, learning, mood, sleep, or movement. The invention also provides methods of identifying an individual having or susceptible to an MCH receptor-associated condition.

10 Such knowledge allows optimal medical care for the individual, including appropriate genetic counseling and prophylactic and therapeutic intervention.

The invention provides a method of identifying an MCH receptor agonist or antagonist. The method 15 consists of contacting an MCH receptor with a candidate compound under conditions wherein the MCH receptor produces a predetermined signal in response to an MCH receptor agonist, and identifying a compound that alters production of the predetermined signal. A compound that 20 alters production of the predetermined signal is characterized as an MCH receptor agonist or antagonist.

As used herein, the term "MCH receptor" refers to a heptahelical membrane-spanning G-protein coupled polypeptide, previously designated SLC-1 or GPR24, which, 25 as disclosed herein, is the endogenous receptor for melanin-concentrating hormone. The term "MCH receptor" encompasses native MCH receptor polypeptides from all vertebrate species including but not limited to human, non-human primate, rat, mouse, rabbit, bovine, porcine, 30 ovine, canine, feline, avian, reptile, amphibian or fish. The human MCH receptor nucleotide sequence (SEQ ID NO:1) and encoded amino acid sequence (SEQ ID NO:2) are described in Kolakowski et al., FEBS Letters 398:253-258

(1996), and are shown in Figure 1. The rat MCH receptor nucleotide sequence (SEQ ID NO:3) and encoded amino acid sequence (SEQ ID NO:4) are described in Lakaye et al., Bioc. Biophys. Acta 1401:216-220 (1998), and are shown in 5 Figure 2. Based on the high degree of identity between rat and human MCH receptor nucleotide and amino acid sequences, it is predicted that MCH receptor from other species will be highly homologous to the rat and human MCH receptor.

10           The term "MCH receptor" also encompasses polypeptides containing minor modifications with respect to a native MCH receptor sequence, and fragments of full-length MCH receptor, so long as the modified polypeptide or fragment retains one or more of the biological 15 activities of a native MCH receptor, such as the ability to selectively bind MCH, or the ability to couple to and signal through a G protein in response to an MCH receptor ligand. A modified polypeptide can have, for example, one or more additions, deletions, or substitutions of 20 natural or non-natural amino acids relative to the native polypeptide, so long as a biological activity of a native MCH receptor is retained.

Furthermore, the term "MCH receptor" encompasses MCH receptor polypeptides as they are found 25 in vertebrate host cells or tissues which express MCH receptor, including but not limited to brain, eye, skeletal muscle, tongue and pituitary, or as they are present in membrane extracts or substantially pure preparations derived from these tissues by standard 30 biochemical fractionation procedures. Additionally, the term "MCH receptor" encompasses recombinantly expressed MCH receptor polypeptides, modifications or fragments, such as recombinant polypeptides expressed in cells or in

cell lysates that support transcription and translation. Methods of producing recombinant polypeptides in cells and lysates are well known in the art. Likewise, the term "MCH receptor" includes chemically synthesized MCH receptor polypeptides, which can be prepared by standard peptide synthesis methods.

The method of identifying an MCH receptor agonist or antagonist is practiced by contacting an MCH receptor with a candidate compound under appropriate conditions in which MCH receptor produces a predetermined signal in response to a known MCH receptor agonist. As used herein, the term "candidate compound" refers to any molecule that potentially acts as an MCH receptor agonist, antagonist or ligand in the screening methods disclosed herein. A candidate compound can be a naturally occurring macromolecule, such as a polypeptide, nucleic acid, carbohydrate, lipid, or any combination thereof. A candidate compound also can be a partially or completely synthetic derivative, analog or mimetic of such a macromolecule or, a small organic molecule prepared by combinatorial chemistry methods. If desired in a particular assay format, a candidate compound can be detectably labeled or attached to a solid support.

Methods for producing pluralities of compounds, including chemical or biological molecules such as simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, nucleic acids, antibodies, and the like, are well known in the art and are described, for example, in Huse et al., U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et al., Med.

Res. Rev. 15:481-496 (1995); and the like. Libraries containing large numbers of natural and synthetic compounds also can be obtained from commercial sources.

The number of different candidate compounds to test in the methods of the invention will depend on the application of the method. For example, one or a small number of candidate compounds can be advantageous in manual screening procedures, or when it is desired to compare efficacy among several identified ligands, agonists or antagonists. However, it is generally understood that the larger the number of candidate compounds, the greater the likelihood of identifying a compound having the desired activity in a screening assay. Additionally, large numbers of compounds can be processed in high-throughput automated screening assays. Therefore, "one or more candidate compounds" can contain, for example, greater than about 10<sup>3</sup> different compounds, preferably greater than about 10<sup>5</sup> different compounds, more preferably, greater than about 10<sup>7</sup> different compounds.

As used herein, the term "MCH receptor agonist" refers to a compound that selectively promotes or enhances normal signal transduction through the MCH receptor. An MCH receptor agonist can act by any agonistic mechanism, such as by binding an MCH receptor at the normal MCH binding site, thereby promoting MCH receptor signaling. An MCH receptor agonist can also act, for example, by potentiating the binding activity of MCH or signaling activity of MCH receptor. The methods of the invention can advantageously be used to identify an MCH receptor agonist that acts through any agonistic mechanism.

As described herein, an example of an MCH receptor agonist is the 19 amino acid MCH cyclic peptide from rat or human having the amino acid sequence shown in Figure 4A (SEQ ID NO:5). A further example of an MCH receptor agonist is the 17 amino acid MCH cyclic peptide from salmon shown in Figure 4A (SEQ ID NO:6). In contrast, somatostatin-14 (Figure 4A, SEQ ID NO:7), the somatostatin analog RC-160, cortistatin-14 (Figure 4A, SEQ ID NO:8), cortistatin-29, MCH-precursor-derived peptide NEI, MCH-gene-overprinted-polypeptide, MGOP-14, MGOP-27, and  $\alpha$ -melanotropin (MSH), as disclosed herein, are not MCH receptor agonists, as they do not promote signaling through the MCH receptor under conditions in which MCH receptor produces a predetermined signal in response to an MCH receptor agonist.

In contrast, as used herein, the term "MCH receptor antagonist" refers to a compound that selectively inhibits or decreases normal signal transduction through the MCH receptor. An MCH receptor antagonist can act by any antagonistic mechanism, such as by binding an MCH receptor or MCH, thereby inhibiting binding between MCH and MCH receptor. An MCH receptor antagonist can also act, for example, by inhibiting the binding activity of MCH or signaling activity of MCH receptor. For example, an MCH receptor antagonist can act by altering the state of phosphorylation or glycosylation of MCH receptor. The methods of the invention can advantageously be used to identify an MCH receptor antagonist that acts through any antagonistic mechanism.

An example of an MCH receptor antagonist is a peptide or peptidomimetic derived from a portion of MCH receptor that binds MCH. In rat MCH receptor, the ligand

binding pocket is predicted to include residues Tyr230, of the fourth transmembrane domain, Phe266, of the fifth transmembrane domain, and Trp318, Tyr322 and Gln325, positioned in the sixth transmembrane domain (Kolakowski et al., FEBS Letters 398:253-258 (1996)). Thus, a peptide or peptidomimetic that includes an MCH receptor amino acid sequence spanning one or more of these residues that constitute the binding pocket of MCH receptor can act as an MCH receptor antagonist.

Suitable assay conditions under which MCH receptor produces a predetermined signal in response to an MCH receptor agonist can be determined by those skilled in the art, and will depend on the particular predetermined signal one intends to detect. As used herein, the term "predetermined signal" refers to a readout, detectable by any analytical means, that is a qualitative or quantitative indication of activation of signal transduction through the MCH receptor. As disclosed herein, MCH receptor couples to G proteins in response to the MCH receptor agonist MCH. Therefore, any known or predicted G protein-coupled cellular event, such as elicitation of second messengers, induction of gene expression or altered cell proliferation, differentiation or viability, can be a "predetermined signal" that is an indication of activation of signal transduction through the MCH receptor.

As used herein the term "G protein" refers to a class of heterotrimeric GTP binding proteins, with subunits designated G $\alpha$ , G $\beta$  and G $\gamma$ , that couple to seven-transmembrane cell surface receptors to transduce a variety of extracellular stimuli, including light, neurotransmitters, hormones and odorants to various intracellular effector proteins. The term "G protein"

encompasses endogenous and recombinantly expressed G proteins from all eukaryotic and prokaryotic organisms, including mammals, other vertebrate organisms, Drosophila and yeast. Also encompassed within the term "G protein" 5 are modifications and fragments of native G proteins that maintain MCH receptor binding activity, signal transduction activity, or both, of a native G protein.

Four major classes of G proteins have been identified, which are defined by their G $\alpha$  subunits, G $\alpha$ i, 10 G $\alpha$ s, G $\alpha$ q and G $\alpha$ 12. As disclosed herein, MCH receptor couples to G proteins containing either G $\alpha$ i and G $\alpha$ q subunits, and potentially couples to G proteins containing other G $\alpha$  subunits. Signaling through G $\alpha$ i-containing G proteins inhibits adenylyl cyclase activity, 15 which can be determined, for example, in an assay that measures increased or decreased forskolin-stimulated cAMP accumulation as the predetermined signal (see Example III, below). Signaling through G $\alpha$ q-containing G proteins promotes calcium ion influx, which can be determined, for 20 example, in an assay that measures an increase or decrease in intracellular Ca $^{2+}$  as the predetermined signal (see Examples I-III, below).

The specificity of G $\alpha$  subunits for cell-surface receptors is determined by the C-terminal five amino acids of the G $\alpha$ . Thus, any convenient G-protein mediated 25 signal transduction pathway can be assayed by constructing a chimeric G $\alpha$  containing the C-terminal residues of a G $\alpha$  known or predicted to couple to MCH receptor, with the remainder of the protein corresponding 30 to a G $\alpha$  that couples to the signal transduction pathway it is desired to assay. As used herein, the term "chimeric G $\alpha$ " refers to any functional G $\alpha$  polypeptide that contains at least the five C-terminal amino acids of

one G $\alpha$ , with the remainder of the polypeptide including amino acid sequences corresponding to one or more different G $\alpha$  subunits.

5       The nucleotide sequences and signal transduction pathways of different classes and subclasses of G $\alpha$  subunits in a variety of eukaryotic and prokaryotic organisms are well known in the art. Thus, one skilled in the art can readily construct any desired chimeric G $\alpha$   
10 by methods known in the art and described, for example, in Conklin et al., Nature 363:274-276 (1993), and Komatsuzaki et al., FEBS Letters 406:165-170 (1995). For example, as described in Example I, below, a chimeric G $\alpha$  that contains amino acids 1-354 of a G $\alpha$ q and the C-  
15 terminal 5 residues of a G $\alpha$ i3 can be constructed by PCR, and used to couple MCH receptor to signaling through the G $\alpha$ q pathway. Likewise, a chimeric G $\alpha$  useful in the methods of the invention can include the C-terminal 5 residues of a G $\alpha$ i and the N terminal residues of a  
20 different G $\alpha$ i, a G $\alpha$ s or a G $\alpha$ 12. As MCH receptor also interacts with G $\alpha$ q (see Example III, below), a chimeric G $\alpha$  useful in the methods of the invention can alternatively include, for example, the C-terminal 5 residues of a G $\alpha$ q and the N terminal residues of a G $\alpha$ i, a  
25 G $\alpha$ s or a G $\alpha$ 12.

Signaling through G proteins containing various G $\alpha$  subunits can lead to increased or decreased production or liberation of second messengers, including, for example, arachidonic acid, acetylcholine, diacylglycerol, cGMP, cAMP, inositol phosphate and ions; altered cell membrane potential; GTP hydrolysis; influx or efflux of amino acids; increased or decreased phosphorylation of intracellular proteins; or activation of transcription. Those skilled in the art can determine an appropriate

assay for detecting alterations in any desired signal transduction pathway in response to a candidate compound. Exemplary assays, including high throughput automated screening assays, to identify alterations in signal 5 transduction pathways and gene expression are described, for example, in Gonzalez et al., Curr. Opin. in Biotech. 9:624-631 (1998) and in Jayawickreme et al., Curr. Opin. Biotech. 8:629-634 (1997), and in references reviewed therein. Yeast cell-based bioassays for high-throughput 10 screening of drug targets for G protein coupled receptors are described, for example, in Pausch, Trends in Biotech. 15:487-494 (1997). A variety of cell-based expression systems, including bacterial, yeast, baculovirus/insect systems and mammalian cells, useful for detecting G 15 protein coupled receptor agonists and antagonists are described, for example, in Tate et al., Trends in Biotech. 14:426-430 (1996).

Assays to detect and measure signal transduction can involve first contacting the cell, 20 extract or artificial assay system with a detectable indicator. Calcium indicators, pH indicators, and metal ion indicators, and assays for using these indicators to detect and measure selected signal transduction pathways are described, for example, in Haugland, Molecular Probes 25 Handbook of Fluorescent Probes and Research Chemicals, Sets 20-23 and 25 (1992-94). Assays to determine changes in gene expression can involve transducing cells with a promoter-reporter nucleic acid construct such that, for example,  $\beta$ -lactamase, luciferase, green fluorescent 30 protein or  $\beta$ -galactosidase will be expressed in response to contacting MCH receptor with an agonist or antagonist. Such assays and reporter systems are well known in the art and are described, for example, at

[http://www.aurorabio.com/tech\\_platform-assay\\_technologies.html](http://www.aurorabio.com/tech_platform-assay_technologies.html).

An assay to determine whether a candidate compound is an MCH receptor agonist or antagonist can be 5 performed either in the presence or absence of a known MCH receptor agonist, such as MCH. Thus, compounds that directly promote or inhibit signaling through MCH receptor, as well as compounds that indirectly affect the normal interaction between MCH receptor and an agonist, 10 or the activity of MCH receptor or an agonist, can be identified by the methods disclosed herein.

The invention also provides compositions useful for identifying MCH receptor agonists and antagonists. In one embodiment, the invention provides a signaling 15 composition containing a recombinantly expressed MCH receptor and a recombinantly expressed G $\alpha$  subunit of a G protein. An example of such a composition is the CHO cell line or HEK 293-T cell line expressing recombinant MCH receptor and recombinant G $\alpha$ q/i3, described in Example 20 II, below. As used herein, the term "signaling composition" refers to any composition in which contacting MCH receptor with an MCH receptor agonist will elicit a predetermined signal.

In another embodiment, the invention provides a 25 signaling composition containing a recombinantly expressed MCH receptor, a G protein, and a calcium indicator. Calcium indicators and their use are well known in the art, and include compounds like Fluo-3 AM, Fura-2, Indo-1, FURA RED, CALCIUM GREEN, CALCIUM ORANGE, 30 CALCIUM CRIMSON, BTC, OREGON GREEN BAPTA, which are available from Molecular Probes, Inc., Eugene Oreg., and described, for example, in U.S. Patent Nos. 5,453,517,

5,501,980 and 4,849,362. An example of a signaling composition containing a recombinantly expressed MCH receptor, endogenous G protein, and a calcium indicator is the CHO cell line expressing recombinant MCH receptor 5 loaded with the calcium indicator Fluo-3 AM, described in Example III, below.

If desired, the  $\text{G}\alpha$  subunit of the G protein in the signaling composition containing a recombinantly expressed MCH receptor, a G protein, and a calcium 10 indicator can be recombinantly expressed. An example of such a composition is the CHO cell line expressing recombinant MCH receptor, recombinant  $\text{G}\alpha\text{q}/\text{i}3$ , and loaded with the calcium indicator Fluo-3 AM, described in Example I, below.

15 As used herein, the term "recombinantly expressed," in reference to an MCH receptor or  $\text{G}\alpha$  subunit of a G protein, refers to a polypeptide that is transiently or stably expressed from a non-natural nucleic acid molecule. Recombinant expression is 20 advantageous in providing a higher level of expression of the polypeptide than is found endogenously, and also allows expression in cells or systems in which the polypeptide is not normally found. A "non-natural" nucleic acid molecule is one that has been constructed, 25 at least in part, by molecular biological methods, such as PCR, restriction digestion and ligation. A non-natural nucleic acid expression construct generally will contain a constitutive or inducible promoter of RNA transcription appropriate for the host cell or 30 transcription-translation system, operatively linked to a nucleotide sequence that encodes the polypeptide of interest. The expression construct can be DNA or RNA, and optionally can be contained in a vector, such as a

plasmid or viral vector. Given knowledge of the nucleic acid sequence encoding MCH receptor and various G $\alpha$  subunits of G proteins, one skilled in the art can recombinantly express these polypeptides using routine laboratory methods, described, for example, in standard molecular biology technical manuals, such as Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992) and Ansuel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1989).

The signaling compositions of the invention include, for example, cells, cell extracts and reconstituted artificial signaling systems. The cell compositions of the invention include any cell type in which MCH receptor can couple to a G protein and induce a detectable signal in response to an agonist, such as a vertebrate cell, insect cell (e.g. *Drosophila*), yeast cell (e.g. *S. cerevisiae*, *S. pombe*, or *Pichia pastoris*) or prokaryotic cell (e.g. *E. coli*). Exemplary vertebrate cells include, but are not limited to, mammalian primary cells and established cell lines, such as COS, CHO, HeLa, NIH3T3, HEK 293-T, PC12, and amphibian cells, such as *Xenopus* embryos and oocytes. Also included are cells from transgenic animals, such as transgenic mice, that have been engineered by known methods to express recombinant MCH receptor or G $\alpha$  subunit.

The signaling compositions of the invention also include crude or partially purified lysates or extracts of the cell compositions of the invention, and reconstituted artificial signaling systems. Artificial signaling systems can include, for example, a natural or artificial lipid bilayer, such as a liposome, to maintain MCH receptor in a natural configuration, and cellular

fractions or isolated components necessary for transducing and detecting the desired predetermined signal.

The invention also provides a method of identifying an MCH receptor ligand. The method consists of contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding between MCH receptor and an MCH receptor ligand. A compound that selectively binds MCH receptor is identified, and the compound is characterized as an MCH receptor ligand.

As used herein, the term "MCH receptor ligand" refers to any biological or chemical compound that selectively binds an MCH receptor polypeptide. An "MCH receptor ligand" can be an agonist or antagonist of MCH receptor, as described above, or can be a compound having little or no effect on MCH receptor signaling, so long as the compound selectively binds an MCH receptor polypeptide. An MCH receptor ligand can be used to specifically target a diagnostic or therapeutic moiety to a region of the brain, or other organ or tissue of the body, that expresses MCH receptor. Thus, an MCH receptor ligand can be labeled with a detectable moiety, such as a radiolabel, fluorochrome, ferromagnetic substance, or luminescent substance, and used to detect expression of MCH receptor polypeptide in an isolated sample or in *in vivo* diagnostic imaging procedures. Likewise, an MCH receptor ligand can be labeled with a therapeutic moiety, such as a cytotoxic or cytostatic agent or radioisotope, and administered in an effective amount to arrest proliferation or kill a cell or tissue that expresses MCH receptor. Thus, an MCH receptor ligand labeled with a therapeutic moiety can be used to treat proliferative

diseases, including cancer and inflammatory diseases, that affect MCH receptor-expressing tissues, or as an alternative to neurosurgery to ablate regions of the brain responsible for MCH receptor-associated conditions,  
5 such as the conditions described below.

An MCH receptor ligand that selectively binds MCH receptor will bind MCH receptor with high affinity, but will not bind, or bind with low affinity, to a structurally related receptor that is not an MCH  
10 receptor, such as a somatostatin receptor. High affinity binding refers to a dissociation constant (Kd) of less than about  $10^{-6}$  M, preferably less than about  $10^{-7}$  M, such as less than about  $10^{-8}$  M. In contrast, low affinity binding refers to a Kd of about  $10^{-4}$  M or more.

15 An example of an MCH receptor ligand is mammalian or salmon MCH which, as disclosed herein, binds and activates MCH receptor with a half-maximal response at nanomolar concentration. A further example of an MCH receptor ligand is an antibody specific for MCH receptor,  
20 such as an antibody specific for an extracellular region of an MCH receptor. In order to prepare an antibody specific for an extracellular region of MCH receptor, a peptide containing substantially the sequence of one of the three extracellular loops of MCH receptor, such as  
25 substantially the sequence HQLMGNGVWHFGETMCT (SEQ ID NO:9), RLIPFPGGAVGCGIRLPNPDTDL (SEQ ID NO:10), QLISISRPTLTTFVY (SEQ ID NO:11), or immunogenic fragment therefrom, or substantially the N-terminal sequence MLCPSTDGSGHSGRIHQETHGEGKRDKISNSEGRENGGRGFQMNGGSLEAEHASRM  
30 SVLRAKPMSNSQRLLLSP (SEQ ID NO:12), or immunogenic fragment therefrom, can be produced, either by direct synthesis, by recombinant means, or by enzymatic digestion of MCH receptor. The peptide can formulated in

an immunogenic composition, such as conjugated to a carrier protein or formulated with an adjuvant, to generate an MCH receptor specific polyclonal or monoclonal antibody using methods well known in the art and described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1989). Methods of preparing fragments of antibodies with specific binding activity, such as Fab fragments, and methods of preparing recombinant, chimeric or humanized antibodies directed against a peptide sequence, are also well known in the art, and such antibodies and fragments directed against MCH receptor are also contemplated as MCH receptor ligands.

15

A variety of low- and high-throughput assays suitable for detecting selective binding interactions between a receptor and a ligand are known in the art. Both direct and competitive assays can be performed, including, for example, fluorescence correlation spectroscopy (FCS) and scintillation proximity assays (SPA) reviewed in Major, J. Receptor and Signal Transduction Res. 15:595-607 (1995); and in Sterrer et al., J. Receptor and Signal Transduction Res. 17:511-520 (1997)). Other assays for detecting binding interactions include, for example, ELISA assays, FACS analysis, and affinity separation methods, which are described, for example, in Harlow and Lane, Eds., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1988). Such assays can be performed, for example, with whole cells that express MCH receptor, membrane fractions therefrom or artificial systems, as described previously, or with substantially purified MCH receptor polypeptide, either in solution or bound to a solid support.

The MCH receptor ligands, agonists and antagonists identified using the methods and compositions of the invention can be isolated and administered to an individual, such as a human or other mammal, in an effective amount to prevent or ameliorate the severity of an MCH receptor-associated condition. As used herein, the term "MCH receptor-associated condition" refers to any pathological condition associated with a tissue or cell in which MCH receptor is expressed. In particular, the term "MCH receptor-associated condition" includes any abnormal physiological or psychological condition in which a quantitative or qualitative alteration in signaling through the MCH receptor contributes to the symptoms of the condition. An MCH receptor-associated condition also includes any physiological or psychological condition in which altering signaling through the MCH receptor has a beneficial effect in the individual.

An MCH receptor-associated condition can have any of a variety of causes, including genetic, environmental and pathological causes. For example, an MCH receptor-associated condition can be caused by a mutation in MCH receptor nucleic acid that alters its expression or structure, a mutation in MCH, or a mutation in a molecule that normally regulates expression or bioavailability of MCH or MCH receptor. An MCH receptor-associated condition can also be caused by environmental factors, such as exposure to toxins, therapeutic drugs or hormones that alter signaling through the MCH receptor, or affect the viability or function of cells or tissues that express the MCH receptor, MCH agonists and antagonists, or their regulatory molecules. An MCH receptor-associated condition can also be due to a pathological condition that affects the viability or

function of cells or tissues that express the MCH receptor, MCH agonists and antagonists, or their regulatory molecules, such as neurodegenerative diseases, infectious diseases, endocrine disorders, and benign and malignant tumors and tumor metastases.

As disclosed herein, MCH receptor is expressed in regions of the brain involved in taste, olfaction, feeding behavior and metabolism. Furthermore, central administration of MCH promotes feeding (Ludwig et al., 10 Am. J. Physiol. 274:E627-E633 (1998)), and MCH mRNA amounts rise as a result of starvation and leptin deficiency (Qu et al., Nature 380:243-247 (1996)). In contrast, MCH deficiency results in reduced body weight and leanness due to reduced feeding and inappropriately 15 increased metabolic rate (Shimada et al., Nature 396:670-674 (1998)). Therefore, MCH receptor-associated conditions can include disorders of body weight and metabolism, including disorders involving increased body weight, such as moderate or severe obesity due to 20 endocrine dysfunction or overfeeding, and disorders involving decreased body weight, such as moderate underweight or cachexia. The term "cachexia" refers to a general weight loss and wasting occurring in the course of a chronic disease, such as cancer or AIDS, or as a 25 result of emotional disturbance, such as anorexia. Thus, an MCH receptor agonist, antagonist or ligand can be used as a drug to restore more normal weight, metabolism and feeding behavior.

As also disclosed herein, MCH receptor is 30 expressed in regions of the brain involved in dopaminergic-modulated responses. Therefore, MCH receptor-associated conditions include pathologies associated with dopamine insufficiency or excess,

including, but not limited to, Parkinson's disease and parkinsonian syndromes, Huntington's disease, and drug- and toxin-induced movement disorders caused by altered availability or activity of dopamine. Thus, MCH receptor 5 agonists and antagonists can be used as therapeutics to prevent or treat conditions due to altered dopaminergic system function.

As further disclosed herein, MCH receptor is expressed in regions of the brain involved in control of behavior, memory and learning, mood and sleep. Disorders 10 of behavior include, but are not limited to, autistic disorder, Asperger's disorder, aggression, pervasive developmental disorders, Tourette's syndrome, attention-deficit hyperactivity disorder and addiction. Disorders 15 of memory and learning include, but are not limited to, Alzheimer's disease; dementia, including dementia due to neurodegenerative diseases, infectious disease, proliferative diseases, endocrine disease, tumors, metabolic disorders, and toxins; and developmental learning disabilities. Disorders of sleep and of the 20 sleep-wake cycle include, but are not limited to, insomnia, bedwetting, sleepwalking, sleep apnea and narcolepsy. Disorders of mood include, but are not limited to, depression; anxiety disorders, such as 25 generalized anxiety disorder, panic attacks, obsessive-compulsive disorder, phobias, acute stress disorder, post-traumatic stress disorder; and psychotic disorders, such as unipolar mania or depression, bipolar disorder and schizophrenia. Thus, MCH receptor agonists, 30 antagonists and ligands can be used as therapeutics to prevent or treat disorders of behavior, memory and learning, mood and sleep.

As further disclosed herein, MCH receptor is expressed in the pituitary, which controls various reproductive functions and developmental growth. Thus, an MCH receptor agonist or antagonist can be used as a  
5 male or female contraceptive, or in treatment of an MCH receptor-associated reproductive disorder, such as male and female sexual dysfunction, impotence, failure of lactation, infertility and precocious puberty, or an MCH receptor-associated growth disorder, such as dwarfism or  
10 acromegaly.

The MCH receptor agonist, antagonist or ligand therapeutics of the present invention can be conveniently formulated for administration together with a pharmaceutically acceptable carrier. Suitable  
15 pharmaceutical carriers for the methods of the invention are well known and include, for example, aqueous solutions such as physiologically buffered saline, and other solvents or vehicles such as glycols, glycerol, oils or injectable organic esters. A pharmaceutical  
20 carrier can contain a physiologically acceptable compound that acts, for example, to stabilize or increase the solubility of a pharmaceutical composition. Such a physiologically acceptable compound can be, for example, a carbohydrate, such as glucose, sucrose or dextrans; an  
25 antioxidant, such as ascorbic acid or glutathione; a chelating agent; a low molecular weight protein; or another stabilizer or excipient. Pharmaceutical carriers, including stabilizers and preservatives, are described, for example, in Martin, Remington's Pharm.  
30 Sci., 15th Ed. (Mack Publ. Co., Easton, 1975).

Those skilled in the art can formulate the therapeutic compounds to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB)

excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes, or chemically derivatized. Those skilled in the art  
5 understand that the choice of the pharmaceutical formulation and the appropriate preparation of the composition will depend on the intended use and mode of administration.

Methods of introduction of a therapeutic  
10 compound of the invention include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral, intranasal, intraspinal and intracerebral routes. Methods of introduction can also be provided by rechargeable or biodegradable devices,  
15 particularly where gradients of concentrations of drug in a tissue is desired. Various slow release polymeric devices are known in the art for the controlled delivery of drugs, and include both biodegradable and non-degradable polymers and hydrogels.

20 An effective dose of a therapeutic composition of the invention can be determined by extrapolation from the concentration required for modulating MCH receptor signaling or binding in *in vitro* assays described herein, and from the dose required for efficacy in an animal  
25 model of the MCH receptor-associated conditions described herein. Typically, an appropriate dose can be in the range of 0.001-100 mg/kg of body weight, but can be determined by those skilled in the art depending on the bioactivity of the particular compound, the desired route  
30 of administration, the gender and health of the individual, the number of doses and duration of treatment, and the particular condition being treated.

The invention also provides methods of identifying an individual having or susceptible to an MCH receptor-associated condition. Such knowledge allows optimal medical care for the individual, including 5 appropriate genetic counseling and prophylactic and therapeutic intervention.

In one embodiment, the method consists of detecting MCH receptor nucleic acid molecule in a sample from the individual. Abnormal structure or expression of 10 MCH receptor nucleic acid molecule in the sample indicates that the individual has, or is at greater risk than a normal individual of developing, an MCH receptor-associated condition.

As used herein, the term "MCH receptor nucleic acid molecule" refers to a DNA or RNA molecule that corresponds to at least a part of a nucleotide sequence of a gene that encodes an MCH receptor. For example, an MCH receptor nucleic acid molecule can be MCH receptor genomic DNA, mRNA, or a nucleic acid molecule derived 20 therefrom, such as a PCR amplification product or cDNA. An MCH receptor nucleic acid molecule can correspond to the sense or antisense strand, and can include coding or non-coding sequence, or both, of an MCH receptor gene. Normal human MCH receptor cDNA has substantially the 25 nucleotide sequence presented in Figure 1 (SEQ ID NO:1), and encodes substantially the amino acid sequence presented in Figure 1 (SEQ ID NO:2).

By detecting MCH nucleic acid in a sample, either altered expression or structure of the nucleic acid molecule can be determined, and used to diagnose or predict risk of developing an MCH receptor-associated condition. As used herein, the term "altered expression" 30

of an MCH receptor nucleic acid molecule refers to an increased or decreased amount of MCH receptor nucleic acid in the test sample relative to levels in a normal sample. Altered abundance of a nucleic acid molecule can 5 result, for example, from an altered rate of transcription, from altered transcript stability, or from altered copy number of the corresponding gene. As used herein, the term "altered structure" of a nucleic acid molecule refers to differences, such as point mutations, 10 insertions, deletions, chromosomal translocations, splice variations and other rearrangements, between the structure of a nucleic acid molecule of the invention in a test sample and the structure of the nucleic acid molecule in a normal sample. Those skilled in the art 15 understand that mutations that alter the structure of a nucleic acid molecule can also alter its expression. Abundance or structure of MCH receptor in a normal sample can, if desired, be determined simultaneously with the test sample, or can be a previously established value.

20 As used herein, the term "sample" refers to any biological fluid, cell, tissue, organ or portion thereof, that is appropriate to detect MCH receptor nucleic acids and polypeptides, and includes samples present in an individual as well as samples obtained or derived from 25 the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid or protein 30 preparation.

The appropriate source and method of preparing the sample can be determined by those skilled in the art, depending on the application of the detection method.

For example, in order to detect structure of MCH receptor genomic DNA, any convenient source of DNA, such as blood cells, lymph cells, cheek cells or skin cells, can be used. However, to detect expression of MCH receptor mRNA or protein, or determine receptor activity, a sample should be obtained from a tissue that expresses MCH receptor, such as neural tissue or, more conveniently, tongue or skeletal muscle.

Various qualitative and quantitative assays to detect altered expression or structure of a nucleic acid molecule in a sample are well known in the art, and generally involve hybridization of a detectable agent, such as a complementary primer or probe, to the nucleic acid molecule. Such assays include, for example, *in situ* hybridization, which can be used to detect altered chromosomal location of the nucleic acid molecule, altered gene copy number, or altered RNA abundance, depending on the format used. Other assays include, for example, Northern blots and RNase protection assays, which can be used to determine the abundance and integrity of RNA; Southern blots, which can be used to determine the copy number and integrity of DNA; SSCP analysis, which can detect single point mutations in DNA, such as in a PCR or RT-PCR product; direct sequencing of nucleic acid fragments, such as PCR amplification fragments; and coupled PCR, transcription and translation assays, such as the Protein Truncation Test, in which a mutation in DNA is determined by an altered protein product on an electrophoresis gel. An appropriate assay format and detectable agent to detect an alteration in the expression or structure of an MCH receptor nucleic acid molecule can be determined depending on the alteration it is desired to identify. Methods of performing such assays are well known in the art.

The invention also provides a method of identifying an individual having or susceptible to an MCH receptor-associated condition, by detecting MCH receptor polypeptide in a sample from the individual. Abnormal 5 structure or activity of MCH receptor polypeptide in the sample indicates that the individual has or is susceptible to an MCH receptor-associated condition.

As used herein, the term "altered expression" of a polypeptide refers to an increased or decreased 10 amount, or altered subcellular localization, of the polypeptide in the test sample relative to known levels or localization in a normal sample. Altered abundance of a polypeptide can result, for example, from an altered rate of translation or altered copy number of the 15 corresponding message, or from altered stability of the protein. Altered subcellular localization can result, for example, from truncation or inactivation of a sorting sequence, from fusion with another polypeptide sequence, or altered interaction with other cellular polypeptides.

20 Various assays to detect altered expression of polypeptides are known in the art, and generally involve hybridization of a detectable agent, such as a labeled ligand, to the polypeptide in a sample, or within the body in diagnostic imaging procedures. Assays to detect 25 altered expression of MCH receptor can be performed *in situ*, in which a detectably labeled ligand, such as an antibody or other ligand identified by the methods described herein, contacts MCH receptor in a whole cell. Other assays to detect altered expression of MCH receptor 30 polypeptide include, for example, ELISA assays, immunoprecipitation, and immunoblot analysis, which can be performed with cell or tissue extracts. An appropriate assay format and detectable agent to detect

an alteration in the expression of MCH receptor polypeptide can be determined depending by those skilled in the art depending on the alteration it is desired to identify. Methods of performing such assays are well known in the art.

Assays to determine activity of MCH receptor have been described above in connection with screening assays to identify MCH receptor agonists and antagonists, and exemplary assays are described in Examples I-III, 10 below. Therefore, one skilled in the art can use such assays to detect qualitatively or quantitatively altered activity of MCH receptor in a sample, compared with normal activity.

The following examples are intended to 15 illustrate but not limit the present invention.

**EXAMPLE I**

**MCH Receptor Assay System**

This example shows an assay system and signaling composition that can be used to identify MCH receptor agonists and antagonists. This assay system was 20 used to identify MCH as an endogenous agonist of MCH receptor.

SLC-1 exhibits about 40% amino acid identity to five known human somatostatin receptors (SSTRs), which are 25 G $\alpha$ -linked receptors. On the assumption that SLC-1 would also bind a peptidic ligand and couple to G $\alpha$ i proteins, different tissues were harvested that were known to express SLC-1, and processed for peptide extraction following protocols described in Reinscheid et al., 30 Science 270:792-794 (1995); Meunier et al., Nature

377:532-535 (1995); and Hinuma et al., Nature 393:272-276 (1998). Initially, chromatographic fractions, prepared as described in Example II, were tested for their ability to induce a decrease in cAMP levels in forskolin-stimulated, SLC-1-transfected CHO cells. None of the fractions showed a response which could be reproducibly followed over several purification steps due to the lability of the cAMP assays.

A new assay system was therefore developed to monitor SLC-1 reactivity through recording of calcium influxes, by forcing SLC-1 to couple to a G $\alpha$ q protein. Because it has been shown that the five C-terminal residues of G $\alpha$  are sufficient for receptor contact, while the rest of the subunit serves to interact with the effector molecule, a G $\alpha$ q/i3 chimera designed to drive SLC-1 to G $\alpha$ q activation was constructed. The G $\alpha$ q/i3 chimera contained the five C-terminal residues of Gai3 (ECGLY) while retaining the rest of the G $\alpha$ q sequence, residues 1-354.

Calcium influx assays were performed in CHO cells transiently cotransfected with the G $\alpha$ q/i3 chimera and SLC-1. Construction of the G $\alpha$ q/i3 chimera by PCR is described in Conklin et al., Nature 363:274-276 (1993), and Komatsuzaki et al., FEBS Lett. 406: 165-170 (1997). The full-length rat SLC-1 cDNA was cloned by PCR using specific oligonucleotides (described in Lakaye et al., Biochem. Biophys. Acta. 1401:216-220 (1997)) from a rat brain Marathon cDNA library (Clontech). The resulting 1.1kb PCR products was subcloned into pcDNA 3.1 (+) expression vector and sequenced. For transient transfection, the SLC-1 cDNA subcloned into pcDNA 3.1 (+) was transfected with the G $\alpha$ q/i3 chimera into CHO dhfr (-) cells using LipofectAMINE PLUS transfection reagent and

following the manufacture's instructions (GIBCO-BRL).

Calcium influx assays were performed as described in Coward et al., Proc. Natl. Acad. Sci. USA 5 95:352-357 (1998). In brief, transfected or control cells were seeded into 96 wells at  $5.5 \times 10^4$  cells/well. The cells were loaded with Fluo-3 AM (Molecular Probes) in standard bath solution (130mM NaCl, 2mM CaCl<sub>2</sub>, 5mM KCl, 10mM glucose, 0.45mM KH<sub>2</sub>PO<sub>4</sub>, 0.4mM Na<sub>2</sub>HPO<sub>4</sub>, 8mM MgSO<sub>4</sub>, 10 4.2mM NaHCO<sub>3</sub>, 20mM HEPES, and 10 μM probenecid) with 0.1% fetal bovine serum for 1 hr at 37°C, then washed with a standard bath solution. Transient changes in [Ca<sup>2+</sup>]<sub>i</sub> evoked by fractions were monitored by the FLIPR system (Fluorometric Imaging Plate Reader, Molecular Devices) in 15 96 well plates at 488 nm for 210 seconds.

As described in Examples II and III below, MCH receptor agonists induce dose-dependent transient increases in cytoplasmic calcium levels in SLC-1-Gαq/i3 transfected cells using the above-described assay system.

20

#### EXAMPLE II

##### Identification of SLC-1 (GPR24) as MCH receptor

This example shows the purification of an endogenous agonist of the orphan G-protein coupled receptor SLC-1 (GPR24) and its identification as the 25 neuropeptide melanin-concentrating hormone (MCH).

The purification of the endogenous SLC-1 ligand was performed as follows. 400g rat frozen brain (Pel-Freez) were extracted in 1M acetic acid and centrifuged 30 at 20,000 x g for 15min at 4°C. The resulting supernatant was precipitated with acetone and extracted with diethylether. The aqueous phase was concentrated and

loaded onto a C18 reverse phase HPLC column (PrepPAK-Delta-Pac 25 x 100mm, Waters) and eluted with a linear gradient of 5-48% CH<sub>3</sub>CN in 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 ml/min. Fractions were monitored for ability to induce increases in [Ca<sup>2+</sup>]<sub>i</sub> in CHO cells transiently cotransfected with the Gαq/i3 chimera and SLC-1, using the calcium influx assay described in Example I, above.

Two consecutive HPLC fractions were identified that elicited a robust increase of cytoplasmic calcium levels in CHO cell cotransfected with the Gαq/i3 chimera and SLC-1, as shown in Figures 3A and 3B. The activities detected in these two fractions, 56 and 57, were specific to the SLC-1-Gαq/i3 system, since they did not induce Ca<sup>2+</sup> influx in cells transfected with ORL-1-Gαq/i3 (Figure 3A). The same fractions also elicited an increase in calcium levels upon cotransfection of SLC-1-Gαq/i3 into HEK 293-T cells.

The increase in calcium levels appeared to be mediated by a peptide in the active fractions, since trypsin treatment abolished activity (see Figure 3B, dotted line). Trypsin treatment was performed by incubating fraction 57 with 20 mU trypsin attached to agarose beads for 3h at 37°C. The reaction was terminated by removing the beads by centrifugation.

Active fractions 56 and 57 from the reverse phase HPLC were pooled and further purified by six more chromatographic steps. Briefly, pooled fractions 56 and 57 were further purified on a cation-exchange column AP-1/SP-8HR (Waters) with a linear gradient of 0.15-0.35M NaCl in 6mM HCl and 30% CH<sub>3</sub>CN. Active fractions were further purified on an analytical C18 Select B column

(Merck) with a linear gradient of 21-33% CH<sub>3</sub>CN in 0.1% TFA. Positive fractions were then serially fractionated in a SMART system on a Sephasil C8 SC2.1/10 column (Pharmacia) with a linear gradient of 36-42% CH<sub>3</sub>CN in 0.1% TFA, a Sephasil C8 SC2.1/10 column (Pharmacia) with a linear gradient of 33-48% CH<sub>3</sub>CN in 0.1% heptafluorobutyric acid (HFBA), on a mRPC C2/C18 SC2.1/10 column (Pharmacia) with a linear gradient of 34.5-35.1% CH<sub>3</sub>CN in 0.1% TFA, and finally on a Sephasil C8 SC2.1/10 (Pharmacia) with a linear gradient of 26.4-27.6.% CH<sub>3</sub>CN in 0.1% TFA at a flow rate of 0.1 ml per minute. The final purification of the active compound by Sephasil C8 SC2.1/10 chromatography is shown in Figure 3C. The inset panel represents the peak increments in [Ca<sup>2+</sup>]<sub>i</sub> induced by designated fractions. The solid line indicates absorption at 214 nm and the dotted line indicates the percentage of CH<sub>3</sub>CN.

The final active compound was subjected to a structural analysis by MALDI mass spectrometry and Edman degradation. Amino acid sequences were determined in a pulse liquid automatic sequencer. Amino acid sequence analysis revealed an N-terminal sequence in which three of the first five residues were identical to that of the rat melanin-concentrating hormone (MCH) described by Vaughan et al., Endocrinology 125:1660-1665 (1989). Synthetic rat MCH was shown to behave identically to the purified active peptide in retention time (by reverse phase HPLC) and in molecular size (by mass data). Therefore, it was inferred that the isolated peptide was MCH. Final yields of MCH was approximately 25pmol/kg of rat brain (wet weight).

To confirm that the isolated peptide had the same activity as MCH, synthetic rat MCH was assayed in

CHO cells cotransfected with the G $\alpha$ q/i3 chimera and SLC-1. Synthetic rat MCH induced a dose-dependent transient increase in [Ca<sup>2+</sup>]<sub>i</sub> in transiently transfected rat SLC-1-G $\alpha$ q/i3 cells (see Figure 4B), but failed to induce detectable [Ca<sup>2+</sup>]<sub>i</sub> changes in mock transfected CHO cells (data not shown). The MCH concentration required to induce half-maximum response (EC50) was calculated to be 4.8 ± 0.5 nM, thus confirming that MCH is an endogenous ligand for SLC-1.

MCH from other species were also tested in SLC-1-G $\alpha$ q/i3 transfected cells. Salmon MCH, described by Kawauchi et al., Nature 305:321-323 (1983), which has a high degree of homology to rat MCH in its central and C-terminal portions, activated SLC-1 with an EC50 of 18.6 ± 2.3 nM. A longer isoform of human SLC-1, described by Kolakowski et al., FEBS Lett. 398:255-258 (1996), was inactive in this assay system.

Because SLC-1 shares sequence similarities with the somatostatin receptors and because somatostatin exhibits a circular topology similar to that of MCH, as shown in Figure 4A, somatostatin-14 and the somatostatin analogue RC-160 were tested on SLC-1-G $\alpha$ q/i3 transfected cells. They were found to be inactive at inducing [Ca<sup>2+</sup>]<sub>i</sub> increase in the cell assay (Figure 4B). Cortistatin-14 or -29, described in De Lecea et al., Nature 381:242-245 (1996), which are structurally related to somatostatin (see Figure 4A), also failed to induce detectable [Ca<sup>2+</sup>]<sub>i</sub> changes (see Figure 4B). Moreover, somatostatin-14 and cortistatin-14 or -29 did not act as antagonists of the MCH-activated SLC-1 response (not shown).

Furthermore, MCH-gene related peptides (MCH-precursor-derived peptide NEI, MCH-gene-overprinted-

polypeptide, MGOP-14, or -27) (Nahonet al., Endocrinology 125:2056-2065 (1989); Toumaniantz et al., Endocrinology 137:4518-4521 (1996)), and  $\alpha$ -melanotropin (MSH) also failed to induce a transient increase in  $[Ca^{2+}]_i$  in 5 transfected SLC-1-Gaq/i3 cells (see Figure 4B). Thus, SLC-1 is a receptor specific for MCH, and consequently the other peptides derived from the MCH gene, if bioactive, must bind to different receptors.

MCH and  $\alpha$ -MSH demonstrate opposite actions on 10 skin coloration in teleost fishes (Baker, Ann. NY Acad. Sci. 680:279-289 (1993)) and exert antagonistic influence on a variety of physiological function including feeding behavior (Miller et al., Peptides 14:1-10 (1993); Gonzalez et al., Peptides 17:171-177 (1996); Sanchez et 15 al., Peptide 18:393-396 (1997); and Ludwig et al., Am. J. Physiol. 274:E627-E633. (1998)). It has also been reported that MCH antagonizes the effect of NEI on grooming and locomotor activities (Sanchez et al., Peptide 18:393-396 (1997)). When tested in the SLC-1- 20 Gaq/i3 transfected cell system at concentrations of 1 nM-1  $\mu$ M, neither  $\alpha$ -MSH nor NEI was able to block the ability of MCH to induce calcium mobilization (data not shown). Since it is known that MCH is not recognized by the melanocortin receptors (Ludwig et al., Am. J. Physiol. 25 274:E627-E633. (1998)), and since it has been demonstrated herein that  $\alpha$ -MSH does not bind the MCH receptor, it can be inferred that the physiological antagonism of these two molecules result from the convergence of signaling pathways activated by distinct 30 receptors.

EXAMPLE IIISignal transduction pathway of MCH receptor

This example shows that MCH receptor couples to G $\alpha$ i- and G $\alpha$ q-containing G protein signal transduction  
5 pathways.

To determine the signaling pathways of SLC-1, a CHO cell line stably expressing stably SLC-1 was established as follows. The SLC-1 cDNA subcloned into pcDNA 3.1 (+) was transfected into CHO dhfr (-) cells by  
10 the calcium-phosphate method described in Saito et al., *J. Neurosci. Res.* 48:397-406 (1997), and stable cell lines were established. To confirm that the plasmid SLC-1 has been integrated into the CHO genomic DNA, these cell lines were analyzed by Northern blot and one clone  
15 was chosen for further experiments.

In these cells, MCH was able to induce robust increases in [Ca<sup>2+</sup>]<sub>i</sub> with EC50 of 18.2 ± 4.6 nM (Figure 4C, left). When G $\alpha$ q/i3 was transiently transfected into these stable SLC-1-expressing cells, the EC50 for MCH on  
20 [Ca<sup>2+</sup>]<sub>i</sub> release was 4.2 ± 0.8 nM (Figure 4C, left), the same value as that found for the SLC-1-G $\alpha$ q/i3 transient cotransfection (Figure 4B).

The effect of MCH on forskolin-stimulated cAMP accumulation was then examined. SLC-1 expressing cells  
25 were plated in 24 well plates and grown to confluence. After removal of the culture medium, variable amounts of synthetic MCH in a total volume 0.3 ml of Dulbecco's modified Eagle's medium [containing 10mM HEPES, 1  $\mu$ M forskolin, and 2  $\mu$ M phosphodiesterase inhibitor Ro20-  
30 1724] were added and the cells were incubated for 15 min at 37°C. The medium was aspirated, the cells were

extracted with 1 ml 70% ethanol, centrifuged to remove the debris, and the supernatant was lyophilized. cAMP content was then measured by competitive binding assay using  $^{125}\text{I}$ -cAMP (NEN).

5           In the stably-transfected SLC-1 cells, MCH potently inhibited forskolin-stimulated cAMP accumulation, showing that SLC-1 can also induce inhibition of adenylyl cyclase. In these experiments, data were normalized to the amounts of cAMP in forskolin-  
10 stimulated cells (100%). All incubations were done in triplicates, with a representative experiment shown in Figure 4C, right. The EC<sub>50</sub> ( $4.1 \pm 1.7 \text{ nM}$ ) for the cAMP assay is similar to that found when the G $\alpha$ q/i3 chimera is expressed.

15           Together, these data indicate that SLC-1 couples not only to G $\alpha$ i but also to G $\alpha$ q, albeit with a lower affinity. The fact that SLC-1 can couple to different G proteins indicates that it may activate different second messenger responses in distinct cellular  
20 environments.

EXAMPLE IV  
Distribution of MCH receptor

25           This example shows the expression of the MCH receptor in mammalian tissues, as determined by Northern blot analysis and *in situ* hybridization.

The 1.1kb insert of SLC-1 was labeled with  $\alpha^{32}\text{P}$ -dCTP and used as a probe in Northern blot analysis. Northern blots containing 3  $\mu\text{g}$  of poly(A)<sup>+</sup> RNA from  
30 various rat tissues were hybridized and washed under high

stringency conditions. Blots were exposed to Kodak X-OMAT film at -80°C with two intensifying screens.

Northern blot analyses of adult rat tissues showed that the 2kb SLC-1 mRNA is detected at a high level in the brain, in moderate amounts in the eye and skeletal muscle and in small amounts in tongue and pituitary (Figure 5A, top panel). Loading was verified by hybridization to a G3PDH control probe (Figure 5A, bottom panel). A possible role for MCH in the eye, skeletal muscle, and tongue has not been thus far investigated. The existence of SLC-1 in the pituitary lends support to a neuroendocrine role for MCH (Ludwig et al., Am. J. Physiol. 274:E627-E633. (1998); Jezova et al., Endocrinology 130:1024-1029 (1992)).

To further localize SLC-1 expression within the central nervous system, *in situ* hybridization was performed using a cRNA probe on rat brain sections. A 0.6 kb BamH1-XbaI fragment of human SLC-1 cDNA was generated and subcloned into the pBluescript II SK (+) vector. The homology between human and rat sequences is 92% in this fragment. Sense and anti-sense riboprobes were generated by T7 and T3 RNA polymerases, respectively, in the presence of 35S-UTP. *In situ* hybridization to adult rat whole brain sections was performed as described by Winzer-Serhan et al., Br. Res. Prots. 3:229-241 (1999). Control hybridization with a sense strand cRNA produced no specific signal (data not shown).

Extensive SLC-1 expression was detected in the hippocampal formation, olfactory regions and the medial nucleus accumbens (Figure 5B, Panels a,b and c). This distribution corresponds to the monosynaptic connections

that MCH neurons make with several areas in the brain involved in integrating inputs related to taste and olfaction (Skofitsch et al., Brain Res. Bull. 15:635-649 (1985); Bittencourt et al., J. Comp. Neurol. 319:218-245 (1992)). This study reveals a possible role of MCH in olfactory learning and reinforcement mechanisms which are fundamental processes in the regulation of feeding behavior. The presence of SLC-1 in the ventromedial nucleus (VMH) of the hypothalamus, a nucleus known to regulate feeding and metabolism (Figure 5B, Panel c) further supports this hypothesis.

Moderate expression of SLC-1 mRNA was found in the substantia nigra, ventral tegmental area and in the amygdala (Figure 5B, Panels b and c), indicating that MCH may modulate the dopaminergic system. Moderate expression of SLC-1 was also detected in the locus coeruleus (Figure 5B, Panel d) which suggests that MCH may participate in the control of various noradrenergic-modulated responses including vigilance, attention, memory, and sleep.

All journal article, reference and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference in their entirety.

25

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, 30 the invention is limited only by the following claims.

What is claimed is:

1. A method of identifying an MCH receptor agonist or antagonist, comprising:

(a) contacting an MCH receptor with one or more candidate compounds under conditions wherein said MCH receptor produces a predetermined signal in response to an MCH receptor agonist; and

(b) identifying a candidate compound that alters production of said predetermined signal, said compound being characterized as an MCH receptor agonist or antagonist.

2. The method of claim 1, wherein said predetermined signal is calcium ion influx.

3. The method of claim 1, wherein said predetermined signal is cAMP production.

4. The method of claim 1, wherein said one or more candidate compounds comprises greater than  $10^5$  compounds.

5. The method of claim 1, wherein said MCH receptor comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or modification or fragment thereof having MCH receptor activity.

6. A method of identifying an MCH receptor ligand, comprising:

(a) contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding between said MCH receptor and an MCH receptor ligand; and

(b) identifying a compound that selectively binds said MCH receptor, said compound being characterized as an MCH receptor ligand.

10 7. The method of claim 6, wherein said one or more candidate compounds comprises greater than  $10^5$  compounds.

8. The method of claim 6, wherein said MCH receptor comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or modification or fragment thereof having MCH receptor activity.

9. A method of identifying an individual having or susceptible to an MCH receptor-associated condition, comprising detecting MCH receptor nucleic acid molecule in a sample from said individual, wherein abnormal structure or expression of said MCH receptor nucleic acid molecule in said sample indicates that said individual has or is susceptible to an MCH receptor-associated condition.

25 10. The method of claim 9, wherein said MCH receptor nucleic acid molecule comprises at least a part of the nucleotide sequence of SEQ ID NO:1.

11. The method of claim 9, wherein said MCH receptor-associated condition is a disorder of body weight, mood, memory, learning, sleep, dopaminergic system function, reproduction or growth.

5 12. A method of identifying an individual having or susceptible to an MCH receptor-associated condition, comprising detecting MCH receptor polypeptide in a sample from said individual, wherein abnormal expression or activity of said MCH receptor polypeptide  
10 in said sample indicates that said individual has or is susceptible to an MCH receptor-associated condition.

13. The method of claim 12, wherein said MCH receptor polypeptide comprises at least a part of the amino acid sequence of SEQ ID NO:2.

15 14. The method of claim 12, wherein said MCH receptor-associated condition is a disorder of body weight, mood, memory, learning, sleep, dopaminergic system function, reproduction or growth.

20 15. A signaling composition, comprising:  
(a) a recombinantly expressed MCH receptor; and  
(b) a recombinantly expressed G $\alpha$  subunit of a G protein.

25 16. The composition of claim 15, wherein said MCH receptor comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or modification or fragment thereof having MCH receptor activity.

30 17. The composition of claim 16, wherein said G $\alpha$  subunit is selected from the group consisting of G $\alpha$ i, G $\alpha$ q and chimeric G $\alpha$ .

18. A signaling composition, comprising:

- (a) a recombinantly expressed MCH receptor;
- (b) a G protein; and
- (c) a calcium indicator.

5 19. The composition of claim 18, wherein said  
MCH receptor comprises the amino acid sequence of SEQ ID  
NO:2 or SEQ ID NO:4, or modification or fragment thereof  
having MCH receptor activity.

10 20. The composition of claim 18, wherein said  
G protein comprises an  $\alpha$  subunit selected from the group  
consisting of G $\alpha$ i, G $\alpha$ q and chimeric G $\alpha$ .

FIG. 1

1/5

1 tccaaacagac agtttctgtc tctgcttcac tcaagaagcc caggctcaga agataccaat  
 61 caaggaaatc cccgcttagga agcctgggtt agggagagct gctggcttg ccagggcaca  
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FIG. 2

2/5

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FIG. 3a

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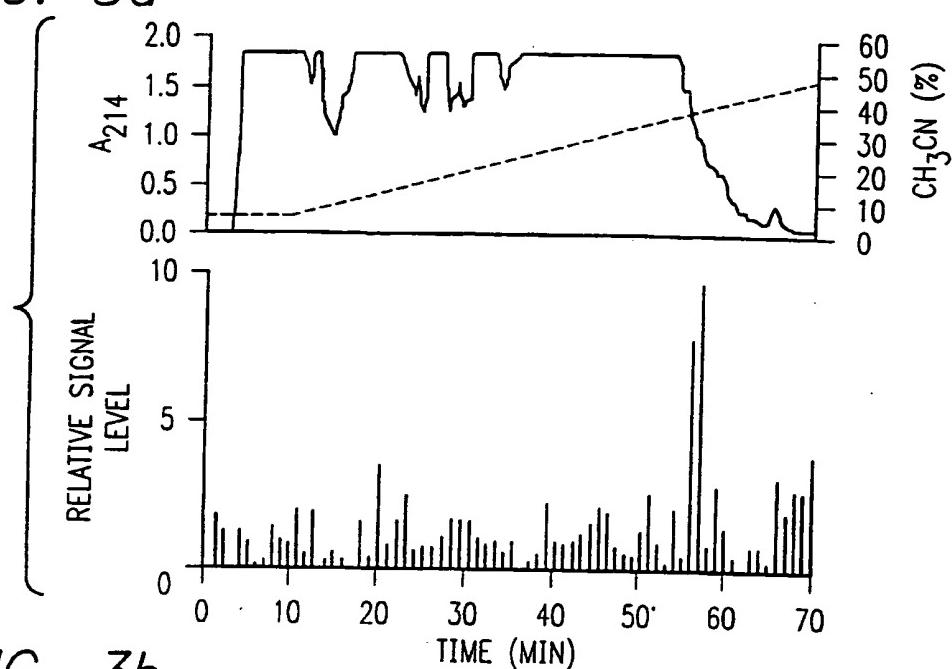


FIG. 3b

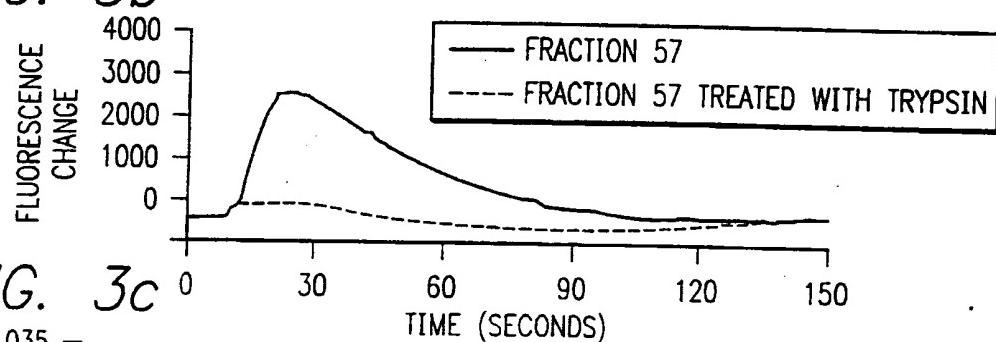


FIG. 3c

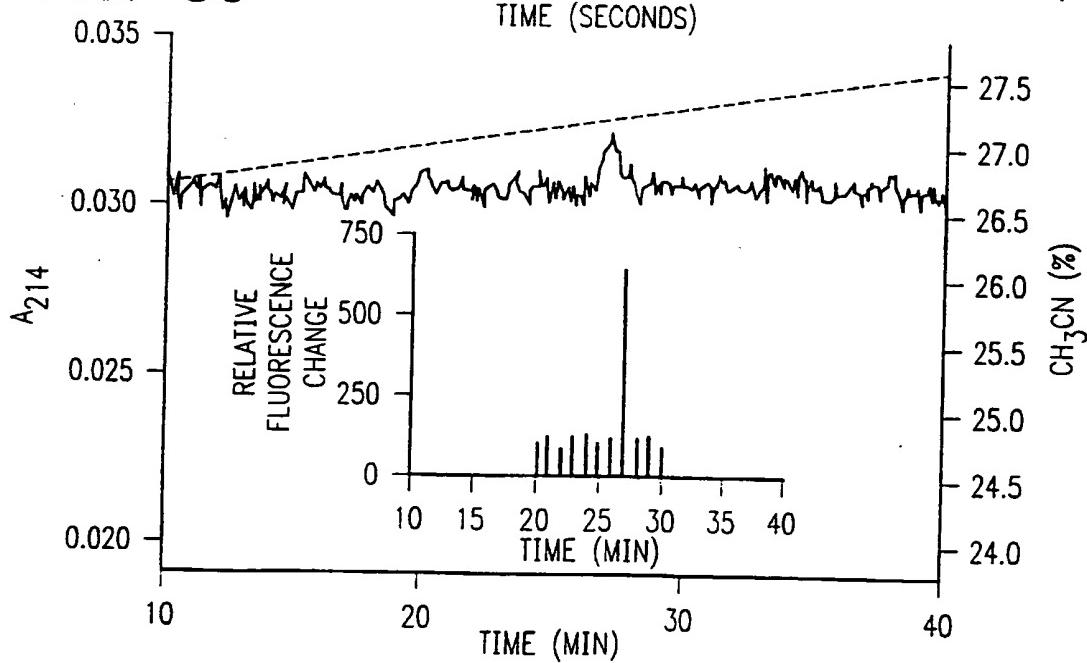


FIG. 4a

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RAT/HUMAN MCH	DFDMLRCMLGRVYRPCWQV
SALMON MCH	DTMRCMVGRVYRPCWEV
SOMATOSTATIN-14	AGCKNFFWKTFSC
CORTISTATIN-14	PCKNFFWKTFSSCK

FIG. 4b

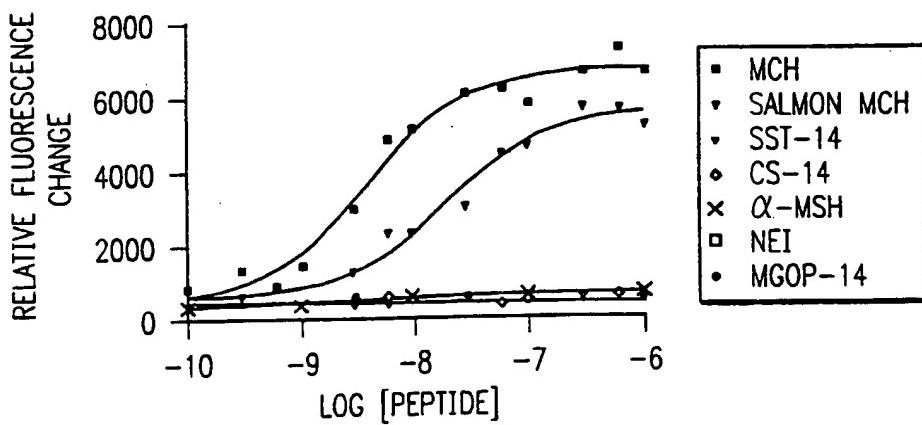
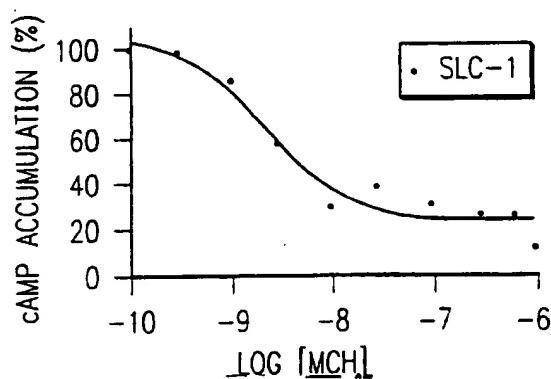
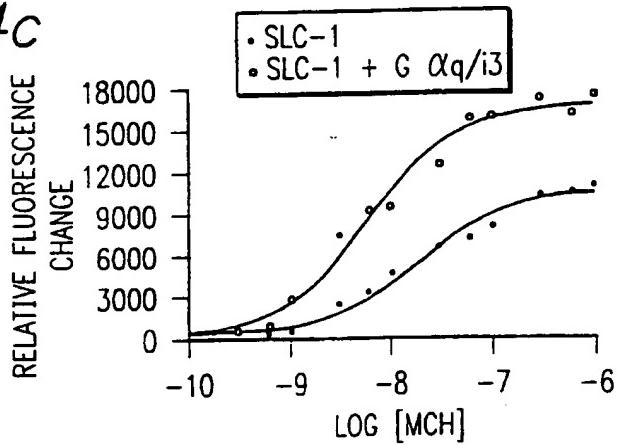


FIG. 4c



5/5

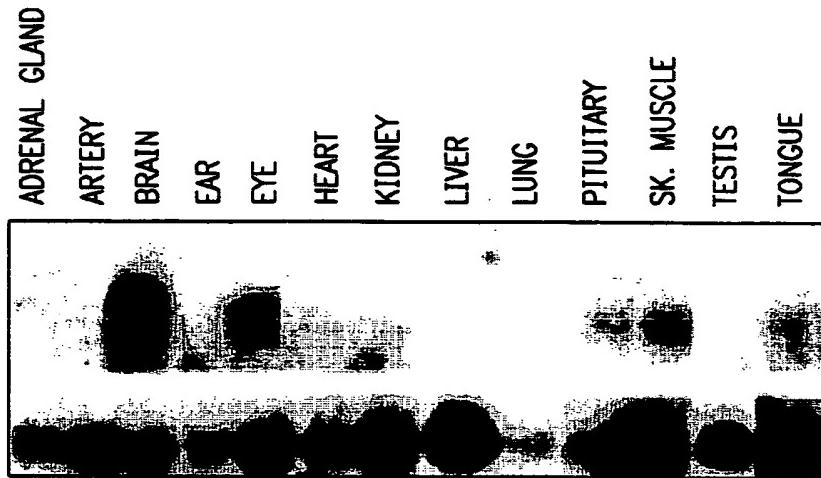
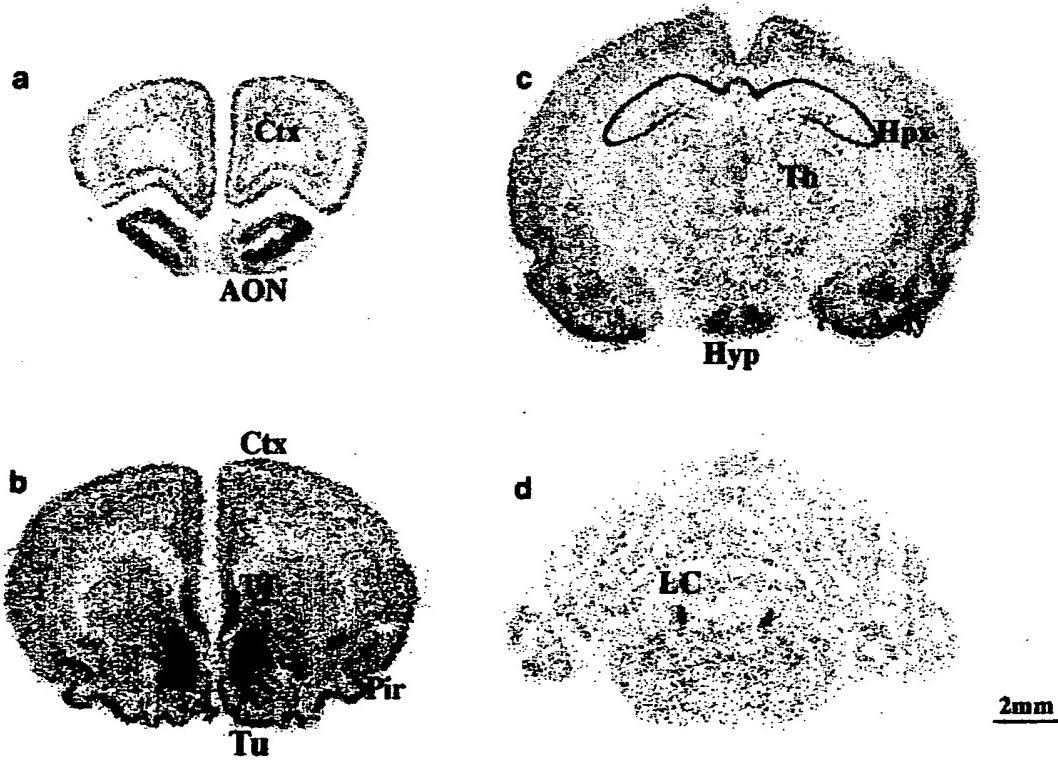


FIG. 5a

FIG. 5b



## SEQUENCE LISTING

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Saito, Yumiko

Nothacker, Hans-Peter

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130 135 140

Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala  
145 150 155 160

Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr  
165 170 175

Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val  
180 185 190

Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe  
195 200 205

Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile  
210 215 220

Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala  
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Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg  
245 250 255

Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr  
260 265 270

Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr  
275 280 285

Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser  
290 295 300

Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys  
305 310 315 320

Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr  
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<212> PRT  
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<400> 8  
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&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly Glu Thr Met Cys

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15

**Thr**

&lt;210&gt; 10

&lt;211&gt; 23

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

Arg Leu Ile Pro Phe Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu

1

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10

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Pro Asn Pro Asp Thr Asp Leu

20

&lt;210&gt; 11

&lt;211&gt; 14

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

Gln Leu Ile Ser Ile Ser Arg Pro Thr Leu Thr Phe Val Tyr

1

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10

&lt;210&gt; 12

&lt;211&gt; 76

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

Met Leu Cys Pro Ser Lys Thr Asp Gly Ser Gly His Ser Gly Arg Ile

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His Gln Glu Thr His Gly Glu Gly Lys Arg Asp Lys Ile Ser Asn Ser  
20 25 30

Glu Gly Arg Glu Asn Gly Gly Arg Gly Phe Gln Met Asn Gly Gly Ser  
35 40 45

Leu Glu Ala Glu His Ala Ser Arg Met Ser Val Leu Arg Ala Lys Pro  
50 55 60

Met Ser Asn Ser Gln Arg Leu Leu Leu Ser Pro  
65 70 75

## INTERNATIONAL SEARCH REPORT

International application No. PCT/US00/15503
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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) :C07J 14/72; C07H 21/04; C12P 21/02; G01N 33/53, 31/00

US CL : 530/350; 536/23.5; 435/69.1, 7.1; 436/6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 435/69.1, 7.1; 436/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 848 060 A2 (SMITHKLINE BEECHAM CORPORATION) 17 June 1998 (17.06.98), see entire document, especially SEQ ID NOS: 1 and 2, abstract, page 3, lines 47-49, page 12, line 11 to page 13, line 4, page 13, lines 22-53, page 14, lines 30-32, page 15, line 37 to page 16, line 35, and page 20, lines 1-19.	1-14
X	WO 96/18651 A1 (SMITHKLINE BEECHAM CORPORATION) 20 June 1996 (20.06.96), see entire document, especially the abstract, SEQ ID NOS: 1 and 2, page 2, line 14-16 and lines 23-29, page 2, line 40 to page 3, line 10, page 10, line 19 to page 11, line 21, page 13, lines 22-38, and page 16, lines 9-21, and claims 14, 17 and 20.	1-14

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search  
29 AUGUST 2000Date of mailing of the international search report  
22 SEP 2000Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No. PCT/US00/15503
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ----	US 6,008,012 A (BERGSMA ET AL) 28 December 1999 (28.12.99), see entire document, especially SEQ ID NOS: 1 and 2, the abstract, column 1, lines 63-67, column 2, lines 9-17 and lines 32-43, column 10, lines 43-65, and column 12, lines 45-61.	1-14 -----
Y, P ----	US 6,033,872 A (BERGSMA ET AL) 07 March 2000 (07.05.00), see abstract, SEQ ID NOS: 1 and 2, column 3, lines 59-63, column 18, lines 27-47, column 19, lines 1-37, column 22, line 45 to column 24, line 6 and column 28, line 36 to column 30, line 2.	15-20 -----
X, P ----	US 6,033,872 A (BERGSMA ET AL) 07 March 2000 (07.05.00), see abstract, SEQ ID NOS: 1 and 2, column 3, lines 59-63, column 18, lines 27-47, column 19, lines 1-37, column 22, line 45 to column 24, line 6 and column 28, line 36 to column 30, line 2.	1-14 -----
Y, P ----	US 6,033,872 A (BERGSMA ET AL) 07 March 2000 (07.05.00), see abstract, SEQ ID NOS: 1 and 2, column 3, lines 59-63, column 18, lines 27-47, column 19, lines 1-37, column 22, line 45 to column 24, line 6 and column 28, line 36 to column 30, line 2.	15-20 -----
A, E	WO 00/39279 A2 (SYNAPTIC PHARMACEUTICAL CORPORATION) 06 July 2000 (06.07.00), see entire document.	1-4

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US00/15503

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

SEQUENCE DATABASES: EST, Issued\_Patents\_NA, N\_Geneseq\_36, GenEmbl, strembl12, swiss-prot38, pir64, a-  
issued, a-geneseq36. SEQ ID NOS searched: 1, 2 and 4.

EAST, STN/CAS, CAPLUS, MEDLINE

search terms: melanin concentrating hormone, receptor, MCH, screen

